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Prognostic Significance of CEA in Colorectal Cancer: A Statistical Study

Clinical evaluation of carcino-embryonic antigen, V

By *J. P. Persijn* and *A. A. M. Hart*

From the Departments of Clinical Chemistry and Medicine, Antoni van Leeuwenhoek-Huis, Netherlands Cancer Institute, Amsterdam, The Netherlands

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Summary: Pretreatment and serial posttreatment carcino embryonic antigen (CEA) serum levels were studied with respect to the course of disease in 222 patients with colorectal cancer. The CEA values during the subsequent six years were expressed in actuarial or cumulative plots in relation to tumour-free period, time of diagnosis of recurrence and other parameters.

The pretreatment CEA value was highly significant but gave no more prognostic information than the *Dukes* classification. The pretreatment CEA had prognostic significance only in inoperable patients. Elevated pretreatment CEA did not exclude the possibility of curative treatment. Normalization of CEA after resection did not indicate completeness of cure.

In patients with local or distant recurrences CEA occasionally rose before recurrences became clinically apparent. Positive lead time was 0–625 days.

However, in about 40% of the patients clinical diagnosis of recurrence preceded a rise of CEA. Maximal negative lead time was 585 days. Statistically, recurrence without a rise of CEA was exceptional. The results stronly suggest that serial CEA determinations cannot replace physical examination and follow-up.

Prognostischer Wert der CEA Bestimmung beim Carcinom des Colon oder Rectum: Eine statistische Untersuchung

Klinische Bewertung des carcinoembryonalen Antigens, 5. Mitteilung

Zusammenfassung: Die Ergebnisse der Bestimmung des carcinoembryonalen Antigens (CEA) bei 222 Patienten mit Carcinom des Colon oder Rectum mit einer Überwachungszeit von bis zu 6 Jahren wurden statistisch bearbeitet.

Die Ergebnisse zeigen, daß dem präoperativ bestimmten CEA signifikant prognostische Bedeutung zukommt. Der präoperative CEA-Wert hat aber keine größere Aussagekraft hinsichtlich der Voraussage einer Rezidivierung oder Metastasierung als die Klassifizierung nach *Dukes*.

Die Behandlung der Patienten mit erhöhtem CEA ist sinnvoll, selbst bei CEA-Werten $> 20{,}0$ µg/l. Ein Abfallen des CEA in den Normalbereich ist kein Beweis für Radikalität der primären Behandlung.

Bei 40% der Patienten stieg der CEA-Wert während der Verlaufskontrolle, nachdem Metastasen oder Lokalrezidive festgestellt wurden. Aus statistischer Sicht gilt, daß bei allen Rezidiven der CEA-Wert schließlich ansteigt, daß aber ein normaler CEA-Wert während der Verlaufskontrolle die Anwesenheit eines sich entwickelnden Rezidivs nicht ausschließt. Fortlaufende CEA-Bestimmungen können übliche klinische Untersuchungsmethoden nicht ersetzen.

Introduction

A precise definition of the clinical role of carcino-embryonic antigen (CEA) in colorectal cancer is possible only when statistical analyses of CEA data in correlation with clinical data in long term follow-up studies are available. There are in fact relatively few studies (1, 2, 3) based on long term follow-up with complete statistical work up, especially concerning the course of CEA in recurrence. Three problems still remain to be solved:

1. does preoperative CEA have additional prognostic significance over histological staging of tumour spread or over other clinical data obtained immediately after primary treatment?

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2. what is the distribution of lead times of CEA before and after objective evidence of recurrence?

3. what is the minimum time for routine follow-up by CEA assays necessary to detect recurrence?

The present report is a statistical analysis of the association of CEA before and after primary treatment with the course of colorectal cancer, in an attempt to answer the above questions. The report concerns 222 patients followed for one to six years. We feel that this report may contribute to final conclusions about the clinical value of CEA.

Patients and Methods

The entire patient population studied consisted of 129 patients with histologically documented colonic cancer and 93 patients with histologically documented cancer of the rectum. The median age was 65 years (30–95 years). The patients of both categories were treated as one group. Two hundred and five patients underwent surgery as primary treatment. In 189 of these patients the surgical treatment was intended to be curative. In the remaining patients surgery was palliative because of distant metastases prior to surgery.

Tumour spread was judged according to *Dukes* classification (4, 5) in 188 patients. Out of these 17 had *Dukes* A, 99 had *Dukes* B and 72 had *Dukes* C lesions. Figure 1 shows the association with rate of recurrence in our patients. Nineteen patients received radiation therapy as primary treatment either because they refused surgery or because they were considered inoperable. Indications of residual tumour after surgery were

(a) cut surfaces of resection material not free of tumour or

(b) tumour remaining in situ after resection or

(c) metastases detected during or prior to palliative surgery.

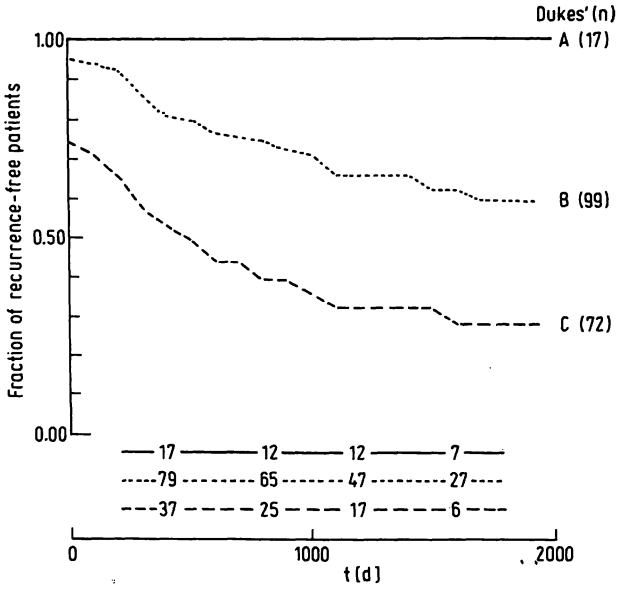

Fig. 1. Actuarial plot of recurrence-free periods for patients divided into *Dukes*'s A, B and C groups. The ordinate indicates percentage of patients remaining recurrence-free after primary therapy. The numbers between parentheses indicate number of patients involved at zero time (time of primary treatment). The numbers below indicate number of patients at risk at the corresponding time interval post therapy. $P = 10^{-9}$.

Indications of residual tumour after radiation therapy or of recurrent disease were: observation of tumour at relaporotomy or colonoscopy, positive findings of liver, or bone scintigram, chest, skeletal or colon X-ray pictures. Other indications were provided by intravenous pyelogram or computer tomography.

Pretreatment CEA values were available in 193 patients. There was no correlation between age and pretreatment CEA ($p = 0.15$).

After primary treatment the patients had routine physical examinations every two to six months. Blood for the CEA test was taken at every examination.

During our study 70 patients out of the group which underwent curative resection showed clinical evidence of recurrence. Among these, local recurrence was demonstrated in 18 patients, metastases in 52 patients.

The CEA assay

CEA was measured in duplicate by a radioimmunoassay as described in full earlier (6). Briefly, the sample is incubated overnight with goat anti-CEA-antiserum and labeled CEA. The addition of the latter is delayed for some hours. The degree of inhibition was measured by the radioactivity in the residue, taking the radioactivity in the absence of unlabeled CEA as 100%. A standard dilution was constructed by adding 16 different known amounts of CEA (from zero to 75.0 µg/l) in human normal serum (from nonsmokers), thus providing sufficient points for the construction of the inhibition curve. The CEA and the goat antiserum were prepared in our laboratory. The lower limit of sensitivity was 3.0 µg/l. Samples resulting in a percentage of bound radioactive CEA lower than 25% over B_0 were re-assayed after appropriate dilution with human normal serum.

Generally, a batch of normal serum was used for three weeks and replaced at the end of this period by a batch taken from another healthy non-smoking donor. However, healthy persons can have different serum CEA values and the change from one batch of normal serum to another may introduce a shift in the measured CEA values. It is evident that for long term follow-up studies such alterations in CEA values must be avoided in a controlable way.

We have solved this problem by introducing a 'standard pool'. The reader is referred to l.c. (6) for further details.

In addition, within every run 4–6 different serum pools were incorporated at various positions scattered throughout the whole series. The range of each pool had been determined by calculation of the mean value and the 95% confidence limits obtained from results within at least 30 runs. New serum pools were only incorporated as controls after their range was established. By these measures sufficient reproducibility for a long period was obtained, which is a prerequisite for long term follow-up studies.

The results from the patients' sera were not accepted if more than one of the serum pools showed values outside established ranges. Comparison of our method with the (Abbott) kit showed a correlation coefficient $r = 0.962$. (Calculated regression line: y (our method) = 1.04 x (Abbott kit) – 0.67).

Statistical analysis

Statistical analysis was done on data accrued over the period before primary treatment till recurrence was clinically manifest, or in recurrence-free patients till termination of the study. Thus, the terms pretreatment and posttreatment refer to primary treatment. The relationship between CEA pretreatment values and prognosis (relapse) was analyzed using the method of *Kaplan & Meier* (7) to produce the graphs, and the test of *Mantel* (8) with the natural numbers as scores for the CEA categories to calculate the p-values. As CEA pretreatment values the last CEA before primary treatment was chosen, but only if this measurement occurred within thirty days before treatment.

To analyse the relationship between serial CEA values after treatment and prognosis, the following definitions have been used. *A CEA fall* was defined by the occurrence of at least two subsequent CEA values satisfying the following criterion: < 5.0 µg/l if the CEA pretreatment value was between 5.0 – 9.9 µg/l, at least

5 μg/l lower than the CEA pretreatment value if this was between 10.0 – 19.9 μg/l or at least 10.0 μg/l lower than the CEA pretreatment value if this was ≥ 20.0 μg/l. Of course, if the CEA pretreatment value was normal, no CEA decrease was observed.

CEA lowest point and lowest value

If a CEA decrease occurred the CEA lowest point was defined by the time at which during this decrease the highest of any two subsequent CEA measurements reached a minimum. This minimum was termed the CEA lowest value. If no CEA decrease occurred, the CEA lowest value was equated to the CEA pretreatment value.

CEA rise

A CEA rise was said to occur when at least two subsequent CEA measurements satisfying the following criterion were noted: if the CEA lowest value was < 5.0 μg/l the subsequent CEA values should be at least 5.0 μg/l; if the lowest value was 5.0 – 9.9 μg/l or 10.0 –19.9 μg/l the subsequent values should be at least 5.0 μg/l higher; if the lowest value was ≥ 20.0 μg/l the subsequent value should be at least 10.0 μg/l higher.

CEA no fall

If neither a CEA decrease nor a CEA increase was observed, the CEA values were termed 'no fall'. These definitions were used so as to minimize on the one hand the influence of occasional outlying points and to retain on the other hand as much resolution as possible.

The statistical significance of the relationship between CEA increase and prognosis was analyzed using a method given by *Clayton* (9) in a different context. This method can be seen to be a generalization of the logrank test: for each time point at which a CEA increase occurred, the prognosis of the patient whose CEA increase did occur on that time was compared, using the logrank test, with the prognosis of patients who, up to that time, had not undergone a CEA increase; these separate comparisons were then combined using the *Mantel-Haenszel* procedure (10). In the case of relapse as a prognostic parameter, a CEA increase occurring after the relapse was discounted in this procedure.

To get a graphic impression of the relationship between CEA increase and prognosis for those patients who, at some time during their follow-up, showed a CEA increase, the intervals between time of CEA increase and time of relapse have been displayed in the way generally used for survival times. In the same graph comparable curves are then given for patients who, within specified intervals from treatment, did not show a CEA increase, measuring relapse time from the end of the specified interval.

Results

Table 1 gives the distribution of CEA pretreatment levels for the different groups according to *Dukes*.

Association of pretreatment CEA with recurrence

Figure 2 is an actuarial plot showing the association of the pretreatment CEA value with subsequent development of disease (local recurrence or distant metastases)

Tab. 1. Pretreatment CEA levels and extent of tumour in 164 patients with colon or rectum cancer.

CEA (μg/l)	< 5.0	5.0 –9.9	10.0 – 19.9	≥ 20.0
	Number of patients within the range shown			
Dukes A	8	2	0	3
Dukes B	27	24	19	19
Dukes C + D	11	11	12	28

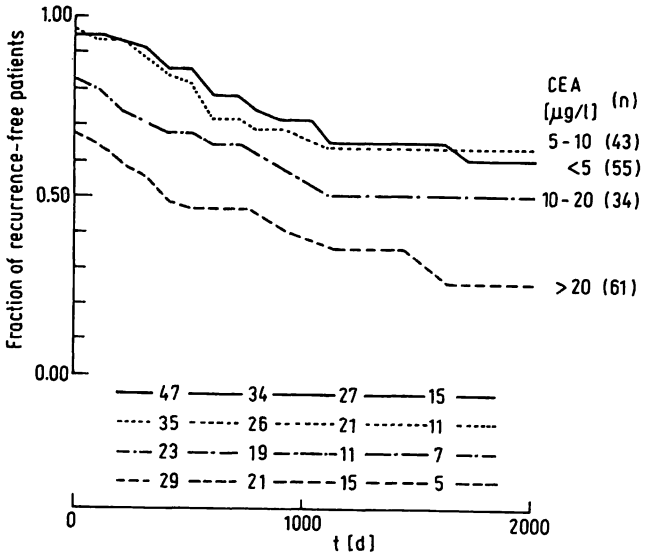

Fig. 2. The association of pretreatment CEA with recurrence in all patients. See legend to figure 1 and section *Patients and Methods* for explanation of presentation. $P = 0.37 \times 10^{-4}$.

in all patients. As can be seen from the intersection of the vertical axis a fraction of approx. 0.30 of the patients with CEA levels ≥ 20.0 μg/l had residual tumour after primary treatment. For patients with pretreatment CEA values between 10.0–19.9 μg/l and < 10.0 μg/l these figures are 0.18 and 0.05 respectively. The prognostic significance of pretreatment CEA is evident: patients with CEA exceeding 10.0 μg/l developed recurrent disease earlier and at a higher rate than those in whom CEA levels were below 5.0 μg/l or between 5.0 and 9.9 μg/l. (The differences between the latter groups were not significant).

Association of pretreatment CEA with recurrence taking posttreatment clinical data into consideration

The patients presented above constituted a heterogeneous group in the sense that 37 patients, i.e. a fraction of 0.16, proved to have residual tumour after primary treatment (see tab. 2). It is interesting to note that after discarding the patients with residual tumour from the analysis the differences between the groups based on their pretreatment CEA values below 19.9 μg/l and ≥ 20.0 μg/l scarcely reached a significant level (fig. 3). Thus, if clinical data obtained during or immediately after primary treatment became available the prognostic value of pretreatment CEA diminished sharply. The prognostic significance of pretreatment CEA was further diminished if the *Dukes* stage was taken into account. Figure 4 shows that tumour-free periods of *Dukes* B patients were not significantly different if grouped according to different levels of their pretreatment CEA values. Similar results were found in patients with *Dukes* C lesions (data not shown). Thus the assessment of CEA prior to surgery provides no additional prognostic information over staging according to *Dukes*.

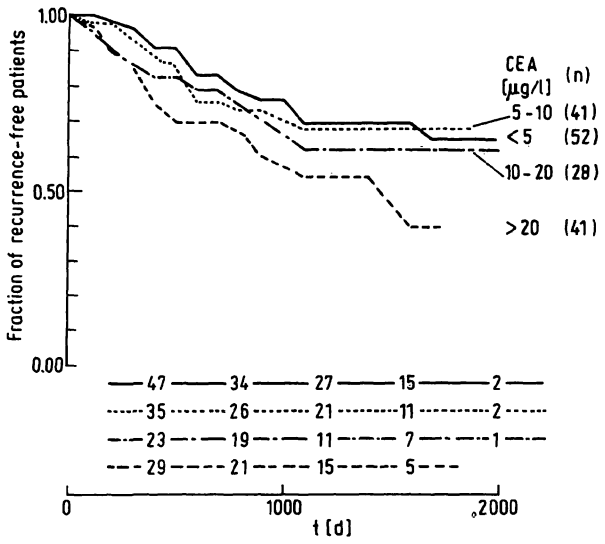

Fig. 3. The association of pretreatment CEA with recurrence in patients without residual tumour. See legend to figure 1 and section *Patients and Methods* for explanation of presentation. P = 0.048.

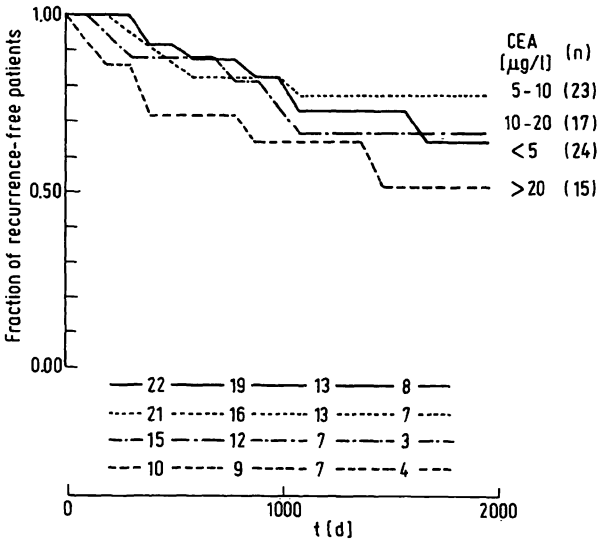

Fig. 4. The association of pretreatment CEA with recurrence in patients with *Dukes* B lesions without metastases. See legend to figure 1 and section *Patients and Methods* for explanation of presentation. P = 0.30.

Fall of posttreatment CEA in patients with residual tumour

Table 2 gives the distribution of the patients according to their pretreatment levels and the presence or absence of residual tumour. In 144 patients with a pretreatment CEA value > 4.9 µg/l the course of posttreatment CEA was known. Among these, 20 patients had residual tumour. It will be noted that posttreatment CEA diminished in 2 patients within 100 days until it was below 5.0 µg/l (even below 3.0 µg/l) in spite of residual tumour. These results indicate the possibility of 'false' decrease of posttreatment CEA.

Tab. 2. Range of CEA values before and after primary therapy

Pretreatment CEA (µg/l)	Residual tumour	No. of patients	Posttreatment CEA < 5.0 µg/l within 100 days		
			Yes	No	Unknown
< 5.0	–	52			
	+	3			
5.0 – 9.9	–	41	11	28	2
	+	2	0	2	0
10.0 – 19.9	–	28	7	20	1
	+	6	0	1	5
> 20.0	–	41	8	31	2
	+	20	1	12	7
not measured	–	23	5	14	4
	+	6	1	3	2

Association of fall of posttreatment CEA with tumour-free period

Figure 5 concerns patients with pretreatment values > 4.9 µg/l and without residual tumour after primary treatment. Fall of serial posttreatment CEA values did not provide any information regarding the duration of the subsequent tumour-free period. From this graph it is apparent that no predictive information is obtained if serial CEA levels decrease.

Association of rise of CEA with subsequent tumour-free period

The bottom line in Figure 6 is an actuarial plot of tumour-free periods following a rise of posttreatment CEA. It should be pointed out that this graph involves only pa-

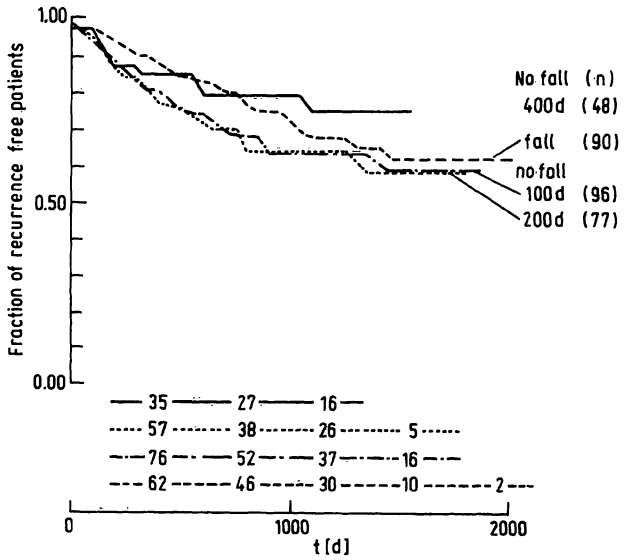

Fig. 5. The association of fall of posttreatment CEA with subsequent tumour-free period. See legend to figure 1 and section *Patients and Methods* for explanation of presentation. P = 0.25.

In the *no fall* curves, time is measured from the end of the indicated interval.

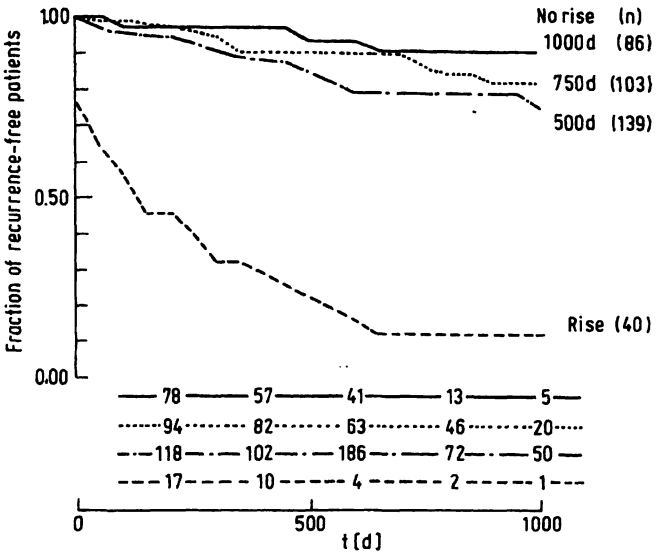

Fig. 6. The association of rise of CEA with subsequent tumour-free period. See legend to figure 1 and section *Patients and Methods* for furtther explanation of presentation. $P < 10^{-8}$.

In the *no rise* curves time is measured from the end of the indicated interval.

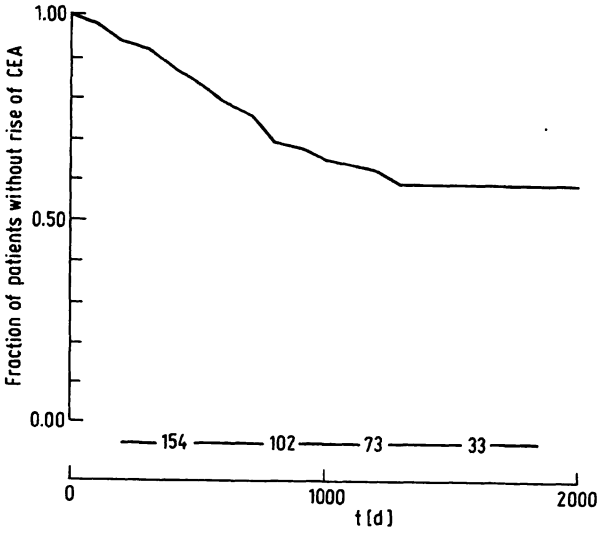

Fig. 7. Relationship of rise of CEA and time from primary therapy. The straight right part of the curve indicates that from 1300 days post therapy the fraction of patients with no rise of CEA remained stationary.

tients with a positive lead time (i.e. CEA rise prior to detection of recurrence). The greatest lead time was 625 days. A fraction of 0.50 of patients showed clinical indications of recurrence within approximately 125 days after CEA rise. On the other hand, as can be seen from the intersection with the vertical axis, a fraction of about 0.22 showed first signs of recurrence at the same time that CEA started to rise. A fraction of 0.30 of patients with CEA rise did not show recurrence. For comparison the upper lines are shown referring to tumour-free periods after a given period in which no rise of CEA (i.e. CEA decrease or CEA no fall) was observed. For example, a fraction of 0.76 of 139 patients, showing no rise of CEA during a posttreatment period of 500 days, were still tumour-free after a further period of 1000 days. Comparison of the data represented by the bottom line and those presented by the upper lines demonstrates that a rise of posttreatment CEA as an indication of recurrence has a prognostic value at a highly significant level. We were therefore interested in defining the minimum follow-up by CEA assays necessary for indication of recurrence. Figure 7 shows that 1300 days after primary treatment no new increase of CEA was observed in our patients. (The last recurrence was observed at day 1611).

Thus, the risk of developing recurrence after a 1300 days period of surveillance without increase of posttreatment CEA can be considered as minimal.

Distribution of lead times in local and distant recurrences

Figure 8 shows a cumulative plot of differences in time of CEA rise in relation to the time of clinical manifestation

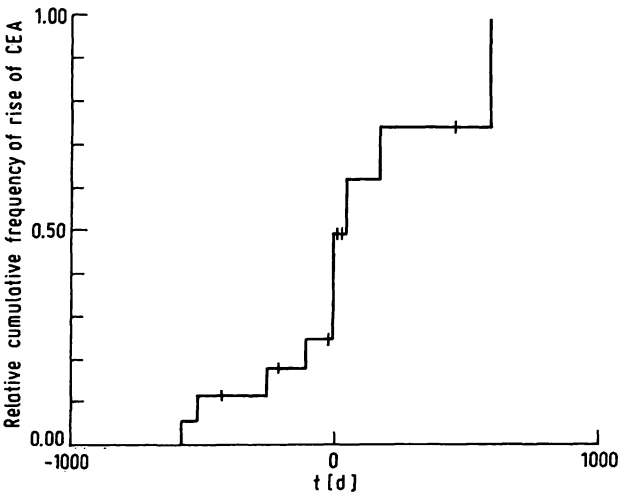

Fig. 8. Cumulative plot of time of CEA rise relative to time of local recurrence (n = 18). Point 0 is time of diagnosis of recurrence. At the negative site (left) of 0 are plotted the positive lead times.

of local recurrence without signs of distant metastases at the time. Positive lead time was observed in a fraction of 0.25 of the patients. On the other hand detection of local recurrence preceded a CEA rise in a fraction of 0.50 of the patients (highest negative lead time was 585 days). A similar distribution of lead times was found in patients with distant metastases (fig. 9). In a fraction of 0.43 of patients the diagnosis of recurrence was preceded up to 625 days by CEA rise. In a fraction of 0.10 the interval between CEA rise and diagnosis of recurrence was at least 275 days, although in a fraction of 0.39 detection of recurrence preceded a rise of CEA (maximal negative lead time 430 days).

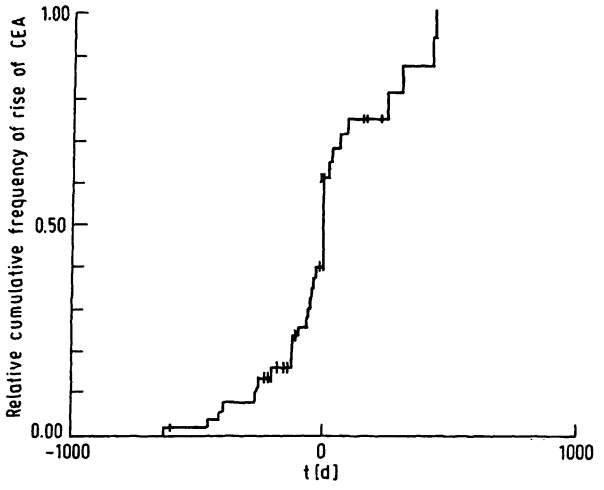

Fig. 9. Cumulative plot of time of CEA rise relative to time of distant recurrence (n = 52). Point 0 is time of diagnosis of recurrence. At the negative site (left) of 0 are plotted the positive lead times.

Discussion

Pretreatment CEA

A role for pretreatment CEA in predicting prognosis is suggested by several data: There is a tendency towards higher fractions of patients with increased CEA from *Dukes* A to *Dukes* C groups (2, 11–14, see also tab. 1). The *Dukes* classification is related to prognosis. We observed that the high prognostic significance of pretreatment CEA (fig. 2) was sharply reduced or even abolished if posttreatment data such as presence of residual tumour or pathological staging became available. Our finding that CEA as a prognostic reference is not superior to staging according to *Dukes* is in agreement with statements of *Beatty* et al (15) and *Evans* et al (1), but contradictory to those of *Herrera* et al (16) and those of *Wanebo* et al (2). CEA has prognostic significance only in inoperable patients.

Decrease of pretreatment CEA to normal is not conclusive for cure (tab. 2). To our knowledge only *Mach* et al (17) have given evidence for the existence of 'false decrease' of pretreatment CEA.

Figure 2 shows that a considerable fractions of patients with elevated pretreatment CEA have recurrence-free periods post therapy up to 2000 days. Thus, elevated pretreatment levels, even if above 20.0 µg/l, do not preclude the possibility of curative treatment.

CEA and recurrence

Figures 5 and 6 give statistical evidence that
a. a decrease or stationary posttreatment CEA does not provide any prognostic information as regards recurrence-free period,

b. a rise of CEA, if defined by strict criteria, is highly prognostic for recurrence,

c. the occurence of a 'false' rise must be taken into account. Such a rise is transient and followed by a return to baseline (15, 18–22).

The curves of figures 8 and 9 indicate that both positive and negative lead times are possible and strongly suggest that a recurrence without any rise of CEA is exceptional (In disseminated breast cancer a different conclusion was reached (24)). This rule seems to hold for both local and distant recurrences. Earlier reports (25, 26, 27) on patients with persisting low CEA values after detection of recurrence can be explained by assuming insufficient time of follow-up by CEA. The limited fraction of patients with positive lead time and data of figures 5 and 6 permit the following observations: a low or normal CEA during follow-up is not a strong indication that the patient is free of recurrence. Serial CEA determinations cannot replace physical examination and symptom review, nor is the converse valid. Recurrence may be detected early in one individual patient by CEA and by physical examination in another. This finding is in agreement with a statement by *Sugarbaker* et al (26). Thirdly, follow-up by CEA can be restricted to about 1300 days (fig. 7) while continuing with physical checks.

The shape of the curves in figures 8 and 9 is related both to frequency of physical examinations and sampling for CEA. The intervals were not standardized. With less frequent examinations, but shorter intervals of CEA tests, the curve would have shifted to the left, resulting in a higher mean value of positive lead time. In the reverse case a higher mean value of negative lead time would have been obtained. Although the precise shape of the curves (figs. 8 and 9) is disputable, conclusions regarding the prognostic function of CEA in detecting recurrence can be made.

Local recurrence is the only type of tumour progression which can be treated curatively. Some authors (21, 28) have studied the potential of CEA rise for early detection of local recurrence, hoping to increase in this way the benefit of secondary surgery. Of all our patients with rising CEA, a fraction of approx. 0.05 had both local recurrence and a minimal positive lead time of 200 days. This low fraction is discouraging. *Staab* et al (21) presented variations of CEA suggesting that a slow rise is indicative for local recurrence. This approach, however still awaits statistical confirmation.

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References

1. Evans, J. T., Mittelman, A., Chu, T. M. & Holyoke, E. D. (1978), Cancer *42*, 1419–1421.
2. Wanebo, H. J., Rao, B., Pinsky, C. M., Hoffman, R. G., Stearns, M., Schwartz, M. K. & Oettgen, H. F. (1978), N. Engl. J. Med. *31*, 448–451.
3. Wood, C. B., Ratcliffe, J. G., Burt, R. W., Malcolm, A. J. H. & Blumgart, L. H. (1980), Br. J. Surg. *67*, 46–48.
4. Dukes, C. E. (1932), J. Pathol. Bact. *35*, 323–332.
5. Dukes, C. E. & Bussey, H. J. R. (1958), Br. J. Cancer *12*, 309–320.
6. Persijn, J. P. & Korsten, C. B. (1976), J. Clin. Chem. Clin. Biochem. *14*, 377–387.
7. Kaplan, E. L. & Meier, P. (1958), J. Am. Statist. Ass. *53*, 457–481.
8. Mantel, N. (1966), Cancer Chemother. Rep. *50*, 163–170.
9. Clayton, D. G. (1978), Biometrika *65*, 141–152.
10. Mantel, N. & Haenszel, W. (1959), J. Natl. Cancer Inst. *22*, 719–748.
11. Jubert, A. V., Talbott, T. M. & Maycroft, T. M. (1978), Cancer *42*, 635–639.
12. Dhar, P., Moore, T., Zamchek, N. & Kupchik, H. Z. (1972), J. Am. Med. Ass. *221*, 31–35.
13. A Joint National Cancer Institute of Canada/American Cancer Society Investigation. (1972), Canad. Med. Ass. J. *107*, 25–33.
14. Martin, E. W., Kibbey, W. E., DiVecchia, L., Anderson, G., Catalano, P. & Minton, J. P. (1976), Cancer *37*, 62–81.
15. Beatty, J. D., Romero, C., Brown P. W., Lawrence, W. & Terz, J. J. (1979), Arch. Surg. *114*, 563–567.
16. Herrera, M. A., Chu, T. M. & Holyoke, E. D. (1976), Ann. Surg. *183*, 5–9.
17. Mach, J. P., Jaeger, Ph., Bertholet, M. M., Ruegsegger, C. H. & Loosli, R. M. (1974), Lancet *II*, 535–540.
18. Booth, S. N., Jamieson, G. C., King, J. P. G., Leonard, J., Oates, G. D. & Dykes, P. W. (1974), Br. Med. J. *4*, 183–187.
19. Sorokin, J. J., Sugarbaker, P. H., Zamcheck, N., Pisick, M., Kupchick, H. Z. & Moore, F. D. (1974), J. Am. Med. Ass. *228*, 49–53.
20. Mach, J. P., Vienny, H., Jaeger, P., Haldemann, B., Egely, R. & Pettavel, J. (1978), Cancer *42*, 1439–1447.
21. Staab, H. J., Anderer, F. A., Stumpf, E. & Fischer, R. (1978), Am. J. Surg. *136*, 322–327.
22. Goldenberg, D. M. (1979), Acta Hepato Gastroenterol. *26*, 1–3.
23. Rittgers, R. A., Steels, G. & Zamcheck, N. (1977), Clin. Res. *25*, 666.
24. de Jong Bakker, M., Persijn, J. P., Hart, A. A. M. & Cleton, F. J. (1981), Eur. J. Cancer, in press.
25. Holyoke, E. D., Chu, T. M. & Murphy, G. P. (1975), Cancer *35*, 830–836.
26. Sugarbaker, P. H., Zamcheck, N. & Moore, F. D. (1976), Cancer *38*, 2310–2315.
27. Mackay, A. M., Patel, S. & Canter, S. (1974), Br. Med. J. *4*, 383–385.
28. Minton, J. P. & Martin, E. W. (1978), Cancer *42*, 1422–1427.

Dr. J. P. Persijn
Ziekenhuis Amsterdam-Noord
NL-1031 XP Amsterdam